Vibrio parahaemolyticus in Thailand

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OBJECTIVE: To determine the characteristics and occurrence of Vibrio parahaemolyticus in Thailand.

BACKGROUND: Vibrio parahaemolyticus, a gram negative, halophilic bacillus was first reported in Thailand in 1970 by the SMRL. Since that time, much attention has been brought to bear on this bacteria's importance as a cause of gastroenteritis in Thailand. In one hospital near Bangkok, this organism has been recovered from up to 25% of the adult patients reporting to that facility with diarrhea. This figure compares with a recovery frequency at that hospital of only 15% of Salmonella spp. and Shigella spp. combined. Other reports have indicated that sea foods from Thailand served to tourists on international flights have harboured V. parahaemolyticus and caused outbreaks of diarrhea and gastroenteritis among the passengers.

The Department of Bacteriology and Mycology has directed its attention to refining certain characteristics of the bacteria, to demonstrating the continued occurence of V. parahaemolyticus in Thai seawaters and sea fish throughout the year, in investigating the Kanagawa phenomenon of this bacteria, and in searching for a model of pathogenicity. Additional effort was expended in a survey of American tourists presenting with diarrhea to a dispensary in a transient billet, in determining antibiotic sensitivity profiles of various isolates of V. parahaemolyticus, and in determiining the frequency of isolation from Thai pediatric patients with diarrhea. A report on a possible model of pathogenicity and on the occurrence of this bacteria in pediatric diarrhea is presented elsewhere in this annual report.

PROGRESS:

CHARACTERISTICS: Vibrio parahaemolyticus was identified in this laboratory by the biochemical and morphological reactions and appearances reported in the 1971 SMRL Annual Report. Extensive serology was attempted using 52 antisera from Japan, however, many of the isolates from Thailand remain untypeable. Of a randomly selected 100 isolates from human diarrhea cultures, 77 were typeable. Only 50% of our isolates from natural sources are typeable. In addition to reactions previously reported, characteristics in arginine, ornithine and lysine decarboxylase media have been noted. Typical reactions in our laboratory reveal that 100% of the isolates failed to decarboxylate arginine, 79.5% exhibited a decarboxylase activity to ornithine and 98.3% were capable of decarboxylating lysine.

Our experience has also noted a need for a careful standardization of methods used to determine the halophilism character of these organisms. One procedure, that of inoculating test tubes of alkaline peptone water (APW) plus varying amounts of NaCl from KIA tubes with 3% NaCl, resulted in a very high percentage of strains growing at 10% NaCl. This characteristic (growth in APW with 10% NaCl) is important as it can often be used to determine the species of other halophilic organisms often associated with V. parahaemolyticus.

Other tests have indicated that numerous strains of V. parahaemolyticus can survive, i.e., be recovered when subsequently streaked on TCBS agar, 18–24 hours at 37C in APW with up to 20% NaCl. If halophilism is measured in this manner—recovery from the test media, then numerous strains would be erroneously classified. Our data indicate that there is seldom any growth (increase in number of organisms/ml of media) at these increased concentrations of NaCl.

Part of the literature fails to mention the use of shaking in testing for halophilism. Our findings indicate that of 60 representative strains in APW with 10% NaCl, 34 demonstrated increased turbidity after 18 hours at 37C in a shaking machine. Only 10% of the 60 strains could produce turbidity after 18 hours at 37C without shaking.

Our results are presently reported as measurements of density in 15 mm screw cap test tubes containing 5 ml of APW with 0%, 3%, 7%, and 10% NaCl. These tubes are inoculated with 1 loopful (3 mm loop) of inocula from a 24 hour culture in APW plus 3% NaCl at 37C. Tubes are then shaken in a 37C water bath for 18 hours and turbidity is visually determined on a 1+ to 4+ scale.

OCCURRENCE: Japanese investigators have reported V. parahaemolyticus is only rarely found in seawaters and seafoods during the winter months. This fact is probably responsible for the up to 70% of summer diarrhea in that country being attributed to this organism. Reports from Viet Nam have also indicated a lower frequency of recovery in the winter months.

In order to determine if there is a fluctuation or an absence of V. parahaemolyticus in Thai sea water or sea foods throughout the year, monthly surveys were conducted during the past year by personnel of SMRL. These surveys consisted of obtaining samples of seawater near the shore line, sand from the shore, and sea fish (mollusks, shrimp, crab, and fish) from local markets. Cultures of fish pastes and sauces that are often used in Thai foods were all negative for V. parahaemolyticus. All of the study sites were near populated areas and the markets are used daily by housewives for purchasing seafoods. Figure 1 presents the data collected since October 1970. The quantity of specimens examined is represented at the left with the shaded portion of the columns depicting those specimens with V. parahaemolyticus. During the first winter that the survey was conducted (Dec 1970; Jan, Feb, 1971) only random samples were collected. The specimens for January 1971, for example, included 65 sand and seawater samples—only 23 of which were positive. In February 1971 only 12 shrimps were examined. More control was exercised in the winter of 71—72 with more uniformity of specimens obtained. These data appear to indicate that there is no decrease in the frequency of recovery of V. parahaemolyticus during those winter months.

Table 1 shows the distribution of isolates during the past 12 months from natural (isolates from sources other than human) sources. It is evident that recovery is more uniform in seafoods than from sand and seawater. Counts of V. parahaemolyticus in sea water obtained along populated shore lines have been between 10,000 and 100,000 organisms per ml. Sand specimens were especially more varied. We suspect this variation is due to water content of the sand, as drier sand specimens usually resulted in fewer positive cultures of V. parahaemolyticus.

Kanagawa Phenomenon:

In 1969, Miramoto and his colleagues reported that strains of V. parahaemolyticus isolated from natural sources failed to haemolyze blood in a special media. In their laboratory, only 0.5% of isolates from sand, seawater, and sea fish haemolyzed red blood cells while 91% of their isolates from human diarrhea patients exhibited haemolysis. This special media, Wagatsuma agar, is made without heat sterilization and contains 5% washed human (group O) or rabbit blood cells. This haemolysis is termed the Kanagawa phenomenon and has been closely associated with pathogenicity because of the above findings.

Isolates of V. parahaemolyticus obtained from natural sources in Thailand do not behave on Wagatsuma agar as do Japanese isolates. Using media made with either rabbit blood cells or human group O cells (blood is washed three times with normal saline and then reconstituted to its original volume with normal saline), this laboratory found that most Thai isolates from either human or natural sources were haemolytic. One hundred twenty-eight isolates from human diarrhea cultures were examined as were 140 from natural sources. Table 2 presents the findings of these experiments. Figure 2 compares this data to that reported from Japan.

The organisms in Japan were tested against rabbit blood cells. Later, personal communication with Dr. R. Sakazaki suggested that we use only human blood in the Wagatsuma agar. Our data reveal that the bacteria from Thailand continued to demonstrate haemolysis in human blood.

Recovery of V. parahaemolyticus from American Tourists:

A survey was conducted at a transient billet (Chao Phya Hotel) in Bangkok to determine the frequency of recovery of V. parahaemolyticus from Americans with diarrhea temporarily residing at that facility. Most of the persons presenting to the dispensary located in the hotel were visiting Bangkok for fewer than 5 days. Most had been in the city for less than 2 days. All test subjects and controls were adult and more than 85% were male. This facility was selected as a study site because it was felt that transients would be less likely to carry their own medication and treat themselves as do many long term residents of Bangkok. Rectal swabs were obtained from the patients, placed in Cary–Blair media immediately, and inoculated onto TCBS agar plates within 6 hours.

Table 3 shows the results of this study. It is noted that 5.6% of the 71 cases studied had V. parahaemolyticus while 4.2% had Salmonella species and only 1 of the 71 had Shigella sonnei isolated from the diarrheal stool. Seventeen American nationals seen at the dispensary for cuts or abrasions were used as controls. Their histories were identical to those patients with diarrhea. None of the 17 control specimens revealed any pathogenic enteric bacteria from stool cultures.

Antibiotic Sensitivities of Various Strains of V. parahaemolyticus:

Numerous strains of V. parahaemolyticus were tested for sensitivities against several commonly used antibiotics. The organisms were grown in BHIB with 3% NaCl at 37C for 20 hours and then seeded onto trypticase soy agar plates. Antibiotic discs were placed on the seeded plates and sensitivity was indicated by zones of inhibition around the discs.

The organisms were divided into groups according to their haemolysis on Wagatsuma rabbit blood agar and the source from which the bacteria were obtained. Group I are strains that are haemolytic, group II are non–haemolytic, group III from human diarrhea, and group IV from sources other than human diarrhea (natural). The results of this study are presented in Table 4.

DISCUSSION: Data obtained in this laboratory indicate differences in V. parahaemolyticus isolated from Thailand's oceans and those organisms from Japan. Japanese bacteriologists continue to base pathogenicity on the Kanagawa phenomenon and thus claim that natural sources are non—pathogenic. Our study of Thai natural isolates indicate that these strains are haemolytic and indistinguishable from those found in diarrheal stools. Our work with the infant rabbit does appear to indicate that natural isolates may be somewhat less pathogenic (possibly less invasive) than isolates from human diarrhea. These data are not, however, as dramatic as the Japanese haemolysis data.

One preliminary experiment conducted in this laboratory may indicate that the haemolytic character of this bacteria may change after several successive passages through animals. Another finding, that 100% of the non—haemolytic strains are sensitive to streptomycin while less than 50% of the haemolytic strains exhibited this sensitivity, may lead to another possible model of pathogenicity.

Certain characteristics of the oceans from which the organisms are obtained may be responsible for some of the differences. Some of our findings suggest that the strains we isolate near populated areas are from fecal contamination. If the organism is physiologically altered by passing through an animal and then dumped back into the ocean, many of our findings would be explained. We are currently examining this and other theses.

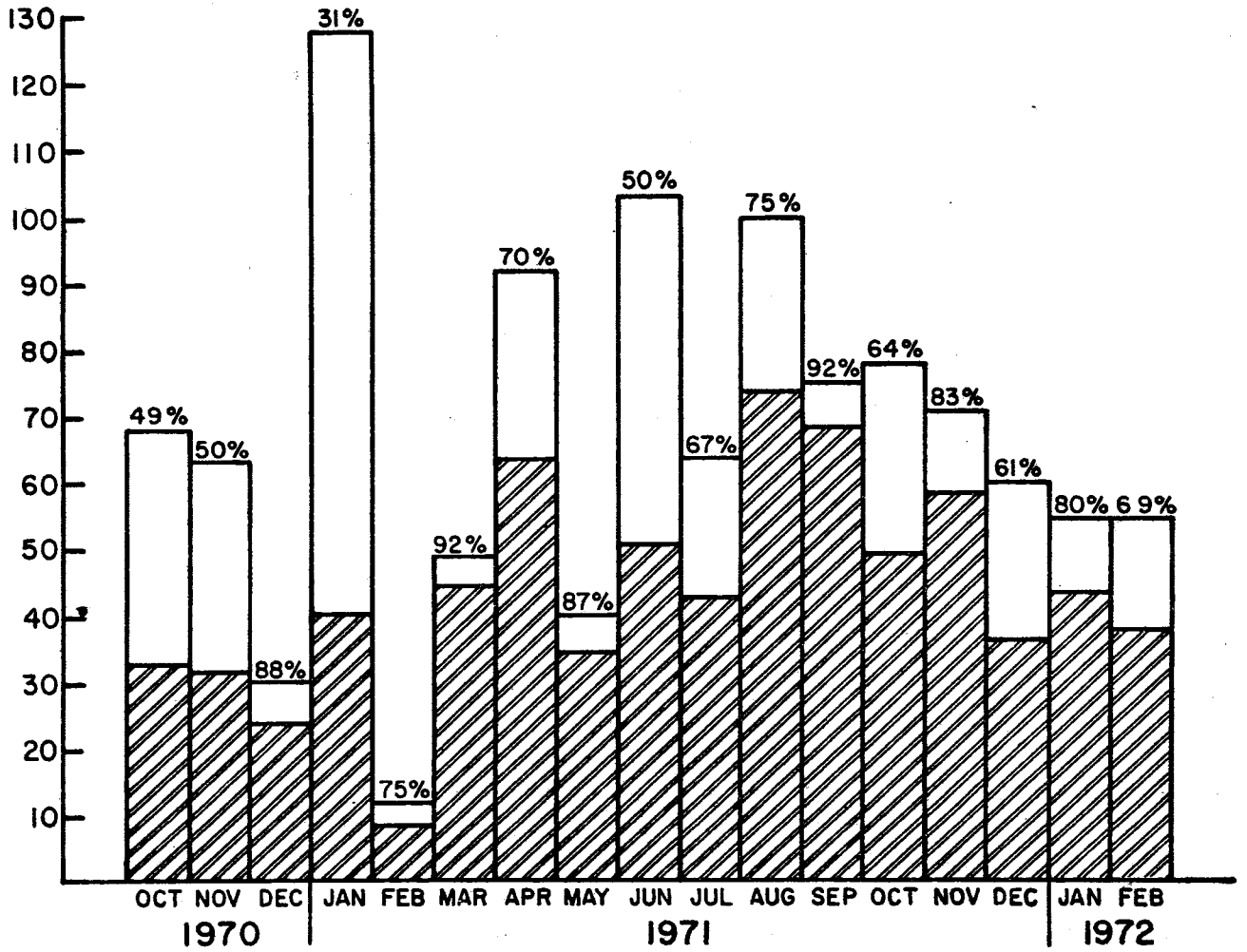

FIGURE I. RECOVERY OF VIBRIO PARAHAEMOLYTICUS FROM NATURAL SOURCES IN THAILAND.

Table 1.

Results of a Twelve Month Survey of Natural Sources for Vibrio parahaemolyticus in Thailand

No. of specimens harbouring Vibrio parahaemolyticus/No. of specimens examined					
Month	Sand & Sea water	Mollusks	Fish	Crab	Shrimp
March 71	0/0	16/18	18/19	9/10	0/0
April	0/0	14/25	14/20	14/14	20/30
May	0/0	13/15	10/20	5/5	7/10
June	25/68	3/10	9/10	5/5	9/10
July	10/29	10/10	8/10	5/5	10/10
August	27/34	10/10	10/15	6/15	10/10
September	4/5	9/10	14/17	8/8	10/10
October	11/19	3/10	9/11	7/8	9/10
November	20/26	16/30	8/10	5/5	10/10
December	9/20	4/10	9/15	5/5	10/10
January 72	14/20	8/10	8/10	5/5	9/10
February	7/20	10/10	7/10	5/5	9/10
March	11/20	7/10	9/15	5/5	8/10

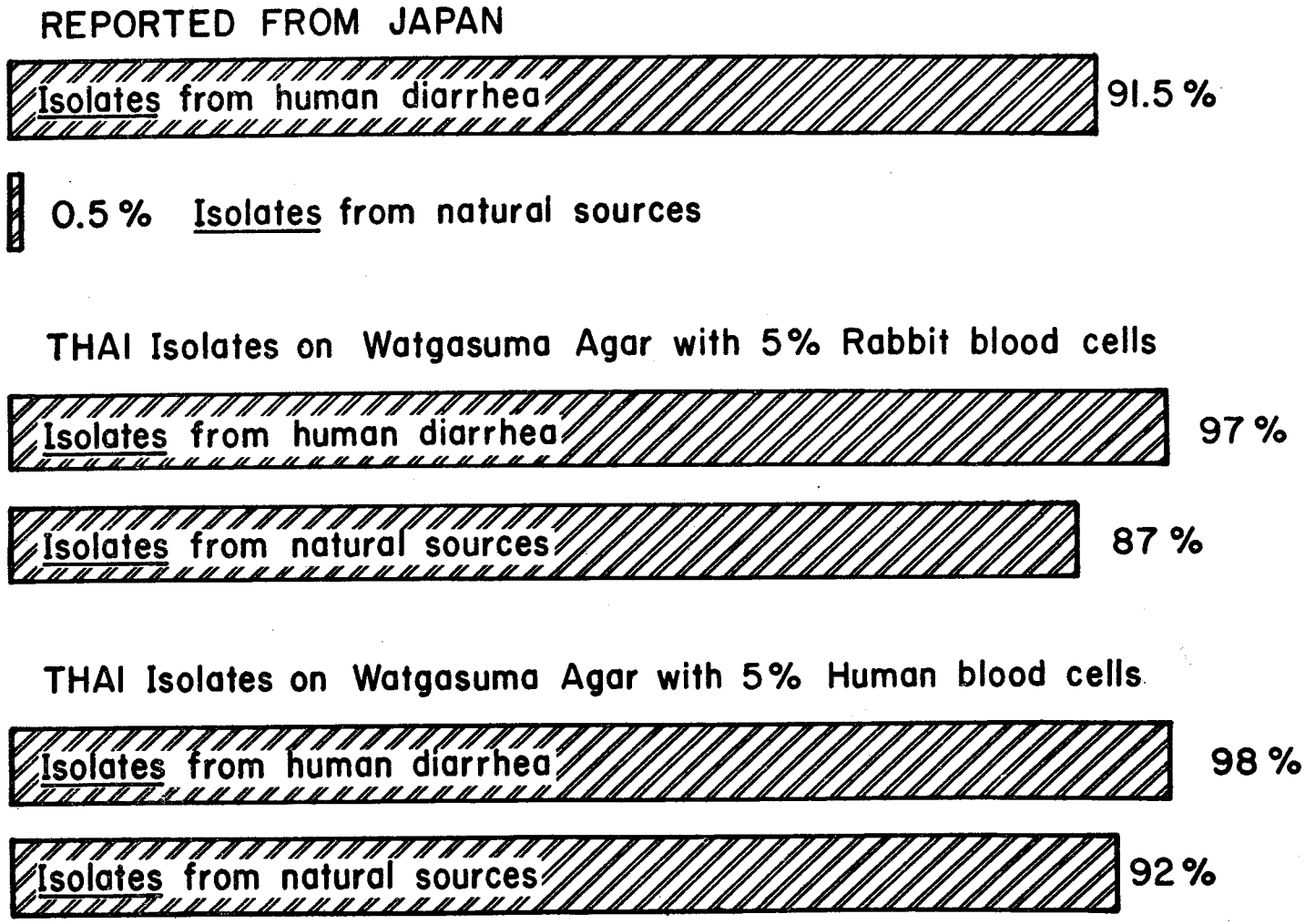

FIGURE 2.
KANAGAWA PHENOMENON OF VIBRIO PARAHAEMOLYTICUS

Table 2.

Kanagawa Phenomenon of Human and Natural Isolates of V. parahaemolyticus

Organisms from:	Haemolytic on Wagatsuma agar with:			
	Human blood		Rabbit blood	
	Number	%	Number	%
Human sources (128)	126	98.4	124	96.8
Natural sources (140)	129	92.1	122	87.1

Table 3.

Enteric Pathogens Isolated from American Nationals with Diarrhea from 29 October 1971 to 15 February 1972

Number of Specimens	Number of patients	Salmonella spp.	Shigella spp.	Vibrio parahaemolyticus
71	71	3	1	4

Table 4.

Sensitivity patterns of Vibrio parahaemolyticus against various antibiotic discs

Antibiotic		Group	# of strains tested	Percent inhibited
penicillin	10 units	I II III IV	67 10 36 41	14.9 % 50.0 % 22.2 % 17.0 %
streptomycin	5 mcg	I II III IV	129 10 98 41	35.6 % 100.0 % 46.9 % 24.3 %
tetracycline	10 mcg	All groups	394	100.0 %
chloramphenicol	10 mcg	All groups	394	100.0 %
Neomycin	10 mcg	All groups	394	100.0 %
Kanamycin	10 mcg	All groups	394	100.0 %
Colimycin	5 mcg	I II III IV	187 10 98 99	92.5 % 80.0 % 93.8 % 89.8 %
Ampicillin	10 mcg	I II III IV	187 10 98 99	32.6 % 40.0 % 44.9 % 21.2 %
Septrin	25 mcg	All groups	394	100.0 %
Furazolidone	150 mcg	All groups	394	100.0 %
Gantricin	1.0 gm	All groups	394	100.0 %